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The effect of puerperal uterine disease on uterine involution in cows assessed by Doppler sonography of the uterine arteries

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1 **The effect of puerperal uterine disease on uterine involution in cows assessed by Doppler**
2 **sonography of the uterine arteries**

3

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ABSTRACT

The objective of this study was to investigate the effects of puerperal uterine disease on uterine blood flow using tran-srectal Doppler sonography. Lactating Holstein Friesian cows ($n = 44$) were divided into two groups based on whether they were healthy (UD–; $n = 23$) or had uterine disease (UD+; $n = 21$) defined as retained fetal membranes and/or metritis. General clinical examination, vaginoscopy, trans-rectal palpation, and trans-rectal B-Mode sonography were conducted on Days 8, 11, 18, 25 and then every 10 days until Day 65 after calving. Doppler sonography of the uterine arteries was conducted on Day 8, during diestrus after the second ovulation (Day 40 to 60 after calving) and during diestrus before breeding (Day 63 to 75 after calving). Cows with uterine disease had greater ($P < 0.05$) uterine size as assessed trans-rectally compared with cows of the UD group. Sonographic measurements on Day 11 after parturition revealed a greater ($P < 0.05$) horn diameter in cows of the UD+ than in the UD– group. Both uterine size and uterine horn diameter decreased more earlier following parturition ($P < 0.05$) in cows of the UD- group. Blood flow volume (BFV) was greater and pulsatility index was less on Day 8 after calving in cows of UD+ than UD– group ($P < 0.05$). In cows of the UD–, but not in those of the UD+group, there was a further reduction in BFV subsequent to Day 45 after calving ($P < 0.05$). The results of this study show that uterine blood flow measures by trans-rectal Doppler sonography are affected by puerperal uterine disease.

Keywords: Uterus; Blood flow; Metritis; Retained fetal membranes; Bovine

1. Introduction

40 Many factors affect fertility in dairy cows. Puerperal uterine diseases including retained fetal
41 membranes (RFM) and metritis are associated with an increase in days from calving to first service
42 and a decrease in conception rate at first service (Fourichon et al., 2000; Elkjaer et al., 2013).
43 Results from studies on the effect of uterine disease on uterine involution are ambiguous.
44 According to some authors, the degree of uterine involution in cows is not affected by RFM and
45 abnormal uterine discharge post-partum (Bosu et al., 1984; Holt et al., 1989), whereas others
46 observed delayed uterine involution in cows with puerperal uterine disease (Fonseca et al., 1983;
47 Mateus et al., 2002). Conventional methods for evaluation of uterine involution such as trans-
48 rectal palpation have limitations because of subjective results (Bekana et al., 1994; Okano and
49 Tomizuka, 1987). B-mode ultrasonography allows objective evaluation of the uterus but not of the
50 entire uterus during the early post-partum period (Aslan et al., 2002; Kamimura et al., 1993).

51 Doppler sonographic evaluation of uterine blood flow has been used for decades to assess
52 uterine involution in women (Jaffa et al., 1996; Nakai et al., 1997; Tekay and Jouppila, 1993).
53 Previous studies in cows evaluated the uterine arteries via Doppler sonography in estrous cycling
54 (Bollwein et al., 2000) and pregnant cows (Bollwein et al., 2002; Panarace et al., 2006) and during
55 the post-partum period (Heppelmann et al., 2012; Krueger et al., 2009). Frequent examinations
56 during the first 2 weeks post-partum revealed a characteristic decrease in uterine blood flow in
57 healthy primiparous cows (Heppelmann et al., 2012). Changes in uterine perfusion until 12 weeks
58 post-partum were recorded in another study with healthy cows (Krueger et al., 2009). The
59 pulsatility index, which measures the resistance in the vascular bed distal to the point of
60 examination underwent distinct changes during the late post-partum period. This led to the
61 conclusion that Doppler sonographic examination of uterine blood flow can serve as an objective
62 method for the description of uterine involution in cows (Krueger et al., 2009). The effects of post-

63 partum uterine disease on uterine blood flow have been described in only one study that was
64 limited to 13 cows examined 2 days after calving (Magata et al., 2013).

65 During the puerperal period, massive amounts of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) were released
66 from the uterus as evidenced in an increase in the metabolite 13, 14-dihydro-15-keto- $PGF_{2\alpha}$
67 (PGFM) in peripheral plasma (Lindell et al., 1982; Kindahl et al., 1999). Cows with a disturbed
68 puerperium attributable to RFM and dystocia had greater PGFM plasma concentrations than
69 healthy cows, (Bosu et al., 1984; Nakao et al., 1997).

70 The main objective of the present study was, therefore, to investigate the effects of post-
71 partum uterine disease on uterine blood flow in dairy cows using trans-rectal Doppler sonography.
72 A secondary goal was to examine the relationship between changes in uterine blood flow and
73 PGFM concentrations.

74

75 **2. Materials and methods**

76 *2.1. Animals*

77 The study was conducted in a herd of 90 lactating Holstein Friesian and Brown Swiss x
78 Holstein Friesian cows at the Research Farm of the University of Veterinary Medicine Hannover,
79 Germany, between February 2009 and July 2010. The cows were housed in a freestall barn and fed
80 a total mixed ration (corn and grass silage with concentrate). Fresh water was available *ad libitum*.
81 The average 305-day milk yield was 10,100 kg. The experimental protocol was approved and
82 conducted in accordance with German legislation on animal rights and welfare (33.9-42502-04-
83 08/1592).

84

85 *2.2. Study design*

Cows were divided into a group of healthy cows without uterine disease (UD–) and a group of cows with RFM and/or metritis (UD+). Fetal membranes were defined as retained when these were not passed within 24 hours after calving. Metritis was diagnosed from Days 4 to 21 (Day 0 = day of calving) by vaginal examination according to a recent study (Sheldon et al., 2009).

On Days 8, 11, 18, 25 and then every 10 days until Day 65 after calving, a general clinical examination, body condition scoring (using a 5-point scale), trans-rectal palpation, vaginoscopy and trans-rectal B-Mode sonography of the internal reproductive organs were conducted. Blood samples were collected weekly. Cows with diseases of other organ systems were excluded from the study. Doppler sonography of the uterine arteries was conducted on Day 8, during diestrus after the second ovulation (Day 40 to 60 after calving) and during diestrus (Day 63 to 75 after calving) before breeding.

For synchronization of time of ovulation, all cows received 0.5 mg of cloprostenol i.m. (Estrumate®, Intervet, Unterschleißheim, Germany) between Days 55 and 60 after calving and 2 days later 0.01 mg of buserelin i.m. (Receptal®, Intervet, Unterschleißheim, Germany). Artificial insemination (AI) was conducted during the subsequent spontaneous estrus. Pregnancy diagnosis was conducted by trans-rectal palpation and sonography 35 to 50 days after AI.

2.3. Trans-rectal palpation and vaginoscopy

During trans-rectal palpation the size of the uterus was assessed. Uterine size was graded according to (Grunert, 1979): uterus retractable and horn diameter <2 cm (score 1), 2 to 5 cm (score 2) or >5 cm (score 3), uterus not retractable but greater curvature palpable (score 4), uterus not retractable and greater curvature incompletely palpable (score 5), and uterus not retractable and greater curvature poorly outlined (score 6).

109 The vulva was cleaned and vaginoscopy was conducted using a vaginal speculum and a
110 flashlight. During the early post-partum period (< 21 days after parturition), the vaginal
111 examination was conducted to diagnose metritis according to Sheldon et al. (2009). After Day 21,
112 the appearance of vaginal discharge was categorized as absent (Score 0), clear mucus (Score 1),
113 containing flecks of pus (Score 2), consisting of < 50% pus (Score 3) and consisting of > 50% pus
114 (Score 4; from Sheldon et al. (2009), modified).

115

116 *2.4. Sonography*

117 All sonographic investigations were conducted using a Powervision SSA-370 ultrasonic
118 machine (Toshiba Co., Tokyo, Japan) equipped with a 7.5 MHz microconvex transducer. Cross-
119 sectional images of the uterine horns were obtained by placing the transducer in a transverse
120 direction. The position of the probe for the examination of the uterine horns was approximately
121 2 cm cranial to the bifurcation. Three images per position were frozen and stored on a Magneto
122 Optical Disc (Sony, Tokyo, Japan). Analysis was conducted off-line on a personal computer using
123 the software FixFoto® (Version 2.74, Joachim Koopmann Software, Wrestedt-Stederdorf,
124 Germany). For further evaluation, the image of the uterus with the greatest contrast and the most
125 circular cross-section was selected from each probe position. The diameter of both uterine horns
126 was estimated. Because the cross-section of the uterine horns was often oval, the diameter was
127 calculated as the mean of the maximum length and width of the endometrium. The ovaries were
128 examined for follicles and corpora lutea.

129 The pulsed-wave mode was used for Doppler sonography of the uterine arteries. Both uterine
130 arteries were identified as described earlier (Bollwein et al., 2000). The uterine artery ipsilateral to
131 the horn where the previous pregnancy occurred is referred to as ipsilateral artery and the uterine
132 artery contralateral to this horn is referred to as contralateral artery. Blood flow waveforms were

133 obtained at an insonation angle of 20 to 60 degrees between the Doppler ultrasonic beam and
134 flow direction. The observations were recorded digitally. After each Doppler sonographic exami-
135 nation, the diameter of both uterine arteries was determined using B-mode sonography. For
136 further evaluations, the means of three measurements of the vessel diameter made during one
137 examination were used. Doppler calculations were completed off-line using the software Pixelflux
138 (Chameleon-Software, Leipzig, Germany). Three figures with two similar consecutive flow velocity
139 waveforms with maximum frequency shifts were selected for each investigation. The uterine
140 blood flow was assessed using the blood flow volume (BFV) and the pulsatility index (PI). The PI
141 represents the ratio of the difference between peak systolic frequency shift (PSF) and minimum
142 diastolic frequency shift (MF) to time-averaged maximum frequency shift over the cardiac cycle
143 (TAMF): $PI = (PSF - MF) / TAMF$. Time-averaged maximum velocity was calculated using the following
144 formula: $TAMV [cm/s] = TAMF [Hz] \times c [cm/s] / (2F [Hz] \times \cos \alpha)$, with c = ultrasonic propagation
145 speed, F = transmitted wave frequency and α = angle between the ultrasound beam and the
146 direction of blood flow. Blood flow volume was computed using the following formula:
147 $BFV [mL/min] = TAMV [cm/s] \times \pi \times (D [cm] \times 0.5)^2 \times 60$ with BFV = blood flow volume [mL/min],
148 D = diameter of the uterine artery [cm] and $TAMV$ = time-averaged maximum velocity [cm/sec].
149 The PI- and BFV-values of the six waveforms were averaged.

150

151 2.5. Blood samples and determination of 13, 14-dihydro-15-keto-PGF₂ α (PGFM)

152 Blood samples were collected weekly (week 1 to 4) from the jugular vein into tubes containing
153 EDTA, and the tubes were immediately placed on ice. After centrifugation (2000 x g, 20 min at 4
154 °C), plasma was harvested and stored at -20 °C until analysis. PGFM was determined using a
155 competitive enzyme immunoassay (Mishra et al., 2003). The PGFM-horseradish peroxidase
156 conjugate and antiserum were supplied by Prof. Meyer (Physiology Unit, Research Center for

157 Nutrition and Food Sciences, Technische Universität München, Freising-Weihenstephan, Germany)
158 and PGFM used for the standard curve was purchased from Sigma, Germany. The antiserum used
159 had minimal cross reactions with any of the related prostaglandins, PGE₂, PGEM, PGA₂, PGAM and
160 PGF₂ (<0.01%, (Mishra and Prakash, 2005)). The minimal PGFM detection limit that significantly
161 differed from 0 was 0.5 pg/20 µl plasma/well, which corresponded to 25 pg/ml plasma. The intra-
162 assay coefficient of variation (CV) was 3.5% and the inter-assay CV was 11.4%.

163

164 2.6. Statistical analysis

165 Statistical analyses were conducted using the Statistical Analysis System V9.3 (SAS Institute,
166 Cary, North Carolina). The Shapiro-Wilk test was used to test for normality of the distribution of all
167 variables. Because all variables had non-normal distributions, the median and the median absolute
168 deviation (MAD) values were given. Differences between the UD+ and UD- groups were analyzed
169 using the non-parametric Wilcoxon's rank sum test (PROC NPAR1WAY). The influence of Day post-
170 partum on variables was determined using the Friedman two-way ANOVA (PROC FREQ).
171 Differences between days within groups were calculated using the Wilcoxon's signed rank test
172 (PROC UNIVARIATE). The χ^2 -test of homogeneity was used to compare categorical data between
173 groups (PROC FREQ). Differences were considered significant at $P < 0.05$.

174

175 3. Results

176 In total, 54 primi- and pluriparous Holstein Friesian cows were used in the study. Five cows
177 were excluded because of diseases of other organ systems (displaced abomasum [$n = 2$], mastitis
178 [$n = 2$] and reticuloperitonitis [$n = 1$]) and another five cows because of technical problems with
179 the ultrasonic machine. This resulted in group sizes of $n = 23$ for UD- and $n = 21$ for UD+. In the
180 UD+ group, four cows (19.1%) had RFM, ten (47.6%) had metritis and seven (33.3%) had RFM and

181 metritis. The cows were 3.7 ± 1.4 years (UD–) and 3.3 ± 1.2 years (UD+) old, parity number was
182 2.0 ± 1.0 and the body condition score (BCS) was 2.75 ± 0.25 for cows of both groups. These
183 variables did not differ ($P > 0.05$) between groups.

184

185 3.1. *Trans-rectal palpation and vaginoscopy*

186 Uterine size was affected by time in both groups ($P < 0.0001$; Fig. 1). Uterine size decreased
187 ($P < 0.0001$) by 80% (UD–) and by 70% (UD+) during the first 45 days. After Day 45, there was no
188 further decrease in the UD– group whereas uterine size decreased by another 10% ($P < 0.05$) in
189 the UD+ group until Day 65. There was a trend for a group effect on uterine size ($P = 0.06$) for the
190 entire examination period. On Days 8, 11 and 18, the cows of the UD+ group had a greater
191 ($P < 0.05$) uterine score.

192 There was no group effect on occurrence of purulent vaginal discharge (Score 2 to 4) for the
193 entire examination period (Day 25 to 65 post-partum; $P > 0.05$). On Days 35 and 45, more
194 ($P < 0.05$) cows of the UD+ group had purulent vaginal discharge than cows of the UD– group
195 (Table 1).

196

197 3.2. *B-mode sonography*

198 The diameter of both uterine horns was affected by time in both groups ($P < 0.0001$, Fig. 2).
199 Between Days 8 and 45, the diameters decreased by 57% (ipsilateral) / 39% (contralateral; UD–;
200 $P < 0.0001$) and 63% / 52% (UD+; $P < 0.05$). After Day 45, there was no further decrease in cows of
201 the UD– group, whereas uterine diameter decreased by another 2% / 5% in cows of the UD+ group
202 until Day 65. There was no group effect on diameter for the entire examination period ($P > 0.05$).
203 On Day 11, uterine diameter was larger in cows of the UD+ than those in the UD– group ($P < 0.05$).

204

205 3.3. Doppler sonography

206 The BFV was affected by time in both groups ($P<0.0001$; Fig 3). During the first 45 days after
207 calving, the BFV decreased ($P<0.05$) by 86% (ipsilateral)/ 68% (contralateral; UD–) and by
208 92% / 95% (UD+). After Day 45, there was no further decrease in BFV in cows of the UD+group,
209 whereas BFV decreased by another 2% / 9% ($P < 0.05$) in cows of UD– group. There was a group
210 effect on BFV in the contralateral artery for the entire examination period ($P<0.05$); on Day 8,
211 cows in the UD+ group had a greater ($P<0.05$) BFV than cows of the UD–group and on Day 65, BFV
212 in the contralateral artery tended to be greater ($P = 0.07$) in cows of the UD+ than in those of the
213 UD– group.

214 With the exception of the contralateral artery in the UD– group, there was a time effect on PI
215 ($P<0.05$; Fig. 4). Between Days 8 and 45, the PI of the ipsilateral artery increased ($P<0.05$) by 54%
216 in cows of the UD– group. During the same time period, the PI of the ipsilateral and contralateral
217 arteries increased ($P<0.05$) by 101% and 70%, respectively, in cows of UD+ group. The PI did not
218 change between Days 45 and 65. There was no group effect on PI for the entire examination
219 period ($P>0.05$). On Day 8, the PI of both arteries was less in cows of UD+ than in those of the UD–
220 group ($P<0.05$).

221

222 3.4. 13, 14-dihydro-15-keto-prostaglandin $F_{2\alpha}$ (PGFM)

223 There was a time effect on PGFM concentration in both groups ($P<0.0001$; Fig. 5). Between
224 Days 7 and 14, PGFM concentrations decreased ($P<0.05$) by 93% and 86% in cows of the UD– and
225 UD+groups, respectively. Afterwards the PGFM-values decreased ($P<0.05$) by a further 1% (UD–)
226 and 6% (UD+) until Day 21. The concentration did not change after Day 21 ($P>0.05$). There was no
227 group effect on PGFM concentrations for the entire study period ($P>0.05$). On Day 21, the PGFM
228 concentration was greater in cows of UD+ than in those of the UD– group ($P<0.05$).

229

230 **4. Discussion**

231 The results of this study revealed that uterine blood flow was affected by puerperal uterine
232 disease. This was evident by an increased BFV and a decreased PI in the uterine arteries of
233 affected cows 8 days post-partum compared with healthy cows. Blood flow volume in the
234 contralateral artery of affected cows was greater than in healthy cows during the entire
235 examination period. This was similar to women with abnormal puerperium because of retained
236 placenta or endometritis, in which uterine vascular resistance was decreased (Kirkinen et al., 1988;
237 Mulic-Lutvica et al., 2009). Uterine involution in cattle is characterized by a reduction in uterine
238 size and vasoconstriction in uterine vessels (Gier and Marion, 1968; Van Camp, 1991). In healthy
239 cows this translates into a decrease in uterine blood flow and increase in vascular resistance
240 within the first 28 days post-partum (Heppelmann et al., 2012; Krueger et al., 2009). The increased
241 BFV in both arteries of cows with uterine disease in the present study can be interpreted to reflect
242 delayed involution, but the causal relationship between the two phenomena is not clear. Some
243 authors suggested that an increased uterine perfusion is responsible for a delayed uterine
244 involution in women (Kirkinen et al., 1988; Sohn et al., 1988). The vasoconstrictive effect of $\text{PGF}_{2\alpha}$
245 (Slama et al., 1991) did not appear to affect blood flow because the PGFM concentration on Day 8
246 did not differ between the two groups. PGE_2 may be involved in the increased uterine blood flow
247 in cows with delayed uterine involution; cows with severe post-partum endometritis had greater
248 PGE_2 concentrations in uterine fluid during the first week post-partum than cows with mild
249 endometritis (Mateus et al., 2003). In addition to an immuno-suppressive effect, PGE_2 has potent
250 vasodilating and myorelaxant action (Slama et al., 1991; Still and Greiss, 1978).

251 Although clinical and histological uterine involution was completed by 40 to 47 days after
252 calving in previous studies (Okano and Tomizuka, 1987; Van Camp, 1991), in the present study it

253 was observed that a further decrease occurred in BFV between Day 45 and 65 in healthy cows. A
254 similar pattern of decreasing uterine perfusion was described in healthy cows studied until Day 86
255 post-partum (Krueger et al., 2009), which indicated that after completion of uterine involution
256 further vascular changes occur. These late vascular changes do not seem to occur in cows with
257 uterine disease because uterine perfusion remained unchanged between Days 45 and 65 in the
258 present study. It appears unlikely that PGE₂ has an effect at this stage of lactation because the
259 PGE₂ concentration in mares with persistent endometritis was not increased compared with
260 healthy mares (Watson et al., 1987). It is possible that inflammatory vascular changes are
261 responsible for incomplete involution of the vascular bed in cows with uterine disease.
262 Histologically, the angiopathies in cows can be divided into angiosclerosis and plevasculitis
263 (Merbach, 2012). Post-partum endometritis in mares is believed to cause vasculitis with
264 endothelial degeneration resulting in severe damage to the vascular wall (Gruninger et al., 1998).
265 Histomorphological investigations of endometrium in infertile and subfertile cows revealed
266 angiopathies in 77% (Rodenbusch et al., 2007).

267 However, in contrast to the results of others (Bosu et al., 1984; Holt et al., 1989), we observed
268 an adverse effect of puerperal uterine disease on uterine involution. While there were distinct
269 differences between the two groups of cows in uterine size during trans-rectal palpation, the
270 sonographic examinations revealed only moderate differences. A possible reason for this is that
271 the sonographic examination was limited to a uterine cross-section near the bifurcation, whereas
272 a much larger part of the uterus was assessed during trans-rectal palpation. As expected and in
273 agreement with another study (Holt et al., 1989), more cows of UD+ group had purulent vaginal
274 discharge during the late puerperal period. This confirmed observations by others that metritis
275 and RFM increased the risk of clinical endometritis (Gautam et al., 2009; LeBlanc et al., 2002).

276 The most pronounced differences between the two groups with respect to uterine size and
277 uterine blood flow variables occurred during the early puerperal period. Interestingly, there was
278 further reduction in uterine size after Day 45 in cows with uterine disease but there were no
279 further changes in uterine blood flow. This suggests that in case of an inflamed uterus, the
280 reduction in uterine size is not necessarily accompanied by vascular involution. It is hypothesized
281 that the inflammatory process affects the vascular system more severely than the connective
282 tissue portion of the uterus, which leads to delayed vascular involution relative to connective
283 tissue involution.

284 Plasma PGFM concentrations were greater on Day 21 and numerically greater on Day 14 post-
285 partum in cows with uterine disease compared with healthy cows. This was in agreement with
286 other studies, in which cows with uterine infection had greater PGFM concentrations (Del Vecchio
287 et al., 1994) and cows with uterine discharge had longer periods of PGFM release after calving
288 (Lindell et al., 1982) than healthy cows. Another study revealed markedly greater PGFM
289 concentrations 1 to 4 days post-partum in cows with dystocia and/or RFM (Nakao et al., 1997), but
290 comparison of those findings with the present study is difficult.

291 In conclusion, uterine blood flow was affected by post-partum uterine disease. Diseased cows
292 had a greater BFV and lesser PI particularly in the early post-partum period. In healthy cows,
293 uterine blood flow further decreased after completion of uterine involution whereas in cows with
294 uterine disease the decrease in uterine size between Days 45 and 65 was not accompanied by
295 further changes in uterine blood flow. These results indicate an association between delayed
296 uterine involution and incomplete regeneration of the uterine vascular bed in cows with puerperal
297 uterine disease.

298

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416

417 **Table**

418

419 Table 1

420 Absolute and relative incidence of purulent vaginal discharge (Score 2 to 4) from Days 25 to
421 65 post partum in dairy cows without (UD–; *n* = 23) and with uterine disease (UD+; *n* = 21).

Days post-partum					
Group	25	35	45	55	65
UD-	13.6%	4.3% ^a	9.1% ^a	4.8%	26.1%
	(3/22)	(1/23)	(2/22)	(1/21)	(6/23)
UD+	38.1%	33.3% ^b	38.1% ^b	10%	9.5%
	(8/21)	(7/21)	(8/21)	(2/20)	(2/21)

422 Within days after calving, values with different letters are different (*P*<0.05).

423

424 Figure legends

425

426 Fig. 1. Changes in uterine size (Scores 1-6) determined by trans-rectal palpation in the first 65 days
427 after parturition in dairy cows without (UD–; $n = 23$) and with uterine disease (UD+; $n = 21$). Values
428 are medians \pm median absolute deviation. Within days after calving, scores with different letters
429 are different between groups UD– and UD+ ($P < 0.05$).

430

431 Fig. 2. Changes in diameter of the formerly pregnant uterine horn determined by ultrasonography
432 in the first 65 days after parturition in dairy cows without (UD–; $n = 23$) and with uterine disease
433 (UD+; $n = 21$). Values are medians \pm median absolute deviation. Within days after calving, values
434 with different letters are different between groups UD– and UD+ ($P < 0.05$).

435

436 Fig. 3. Changes in blood flow volume (BFV) in the ipsilateral and contralateral arteries in the first
437 65 days after parturition in dairy cows without (UD–; $n = 23$) and with uterine disease (UD+;
438 $n = 21$). Values are medians \pm median absolute deviation. For both arteries within days after
439 calving, values with different letters are different between groups UD– and UD+ ($P < 0.05$). Values
440 with an asterisk differ from corresponding previous values ($P < 0.05$).

441

442 Fig. 4. Pulsatility index (PI) of the ipsilateral and contralateral arteries in the first 65 days after
443 parturition in dairy cows without (UD–; $n = 23$) and with uterine disease (UD+; $n = 21$). Values are
444 medians \pm median absolute deviation. For both arteries within days after calving, values with
445 different letters are different between groups UD– and UD+ ($P < 0.05$). Values with an asterisk
446 differ from corresponding previous values ($P < 0.05$).

447

448 Fig. 5. Plasma PGFM concentrations in the first 28 days after parturition in dairy cows without
449 (UD-; $n = 23$) and with uterine disease (UD+; $n = 21$). Values are medians \pm median absolute
450 deviation. Within days after calving, values with different letters are different ($P < 0.05$). Values
451 with an asterisk differ from corresponding previous values ($P < 0.05$).

452

453